

# Quantitative aspects of chlamydial infection of the cervix

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**SUMMARY** In 580 women with *Chlamydia trachomatis* infection of the cervix, the degree of the infection was assessed by counting the number of chlamydial inclusions which developed in McCoy cell monolayers inoculated with cervical swab material under standardised conditions.

In 34% of these women inclusion counts were <100 per monolayer whereas in 36% the counts were ≥1000. Clinical features in each of these groups were compared to see if certain factors could be identified as the cause, or the result, of high-grade rather than low-grade infection.

A significant association was found between high inclusion counts and the presence of cervical mucopus or cervical ectopy. Oral contraceptives acted additively with ectopy but had no significant effect alone. Concurrent gonorrhoea did not affect the degree of chlamydial infection. High inclusion counts were more common in women under 20 years of age than in older women.

## Introduction

Over the past five years, our combined clinical and laboratory investigations of women with genital tract infections with *Chlamydia trachomatis* have shown large variations between individual infected patients, both in the number and severity of clinical signs<sup>1</sup> and in the number of chlamydial inclusions obtained from cervical swabs inoculated into McCoy cell tissue cultures.<sup>2</sup> In a group of 202 sexual contacts of men with nongonococcal urethritis (NGU), women who had cervical mucopus, or those who had cervical ectopy and were also using oral contraceptives, showed a significantly higher prevalence of chlamydial infection than did women without these clinical features.<sup>3</sup> It seemed of interest to determine if these or other clinical factors might be correlated not only with a high rate of infection but with a more severe infection than in women in whom these factors were absent.

In McCoy cell tissue cultures infected with *C. trachomatis* only a single cycle of development of the agent occurs, and each intracellular inclusion found after incubation represents a single infective chlamydial particle in the original inoculum. Thus, when standard amounts of material from cervical

swabs are inoculated and incubated under constant conditions in coverslip monolayers of McCoy cells the final total number of inclusions found over the whole cell sheet should give a direct quantitative measure of the number of infective chlamydial particles in the cervical secretions of each patient.

The sensitivity and reproducibility of the laboratory method we have used has been investigated previously;<sup>2,4</sup> for example, in 48 McCoy cultures inoculated with a single sample of *C. trachomatis* the counts on individual coverslips varied from the mean by a maximum of only ±23%, and, when a second swab was taken seven days after the first swab from 106 untreated infected women, the inclusion counts were of the same magnitude in both swabs in 92.5% of cases. Thus, the wide variations in inclusion counts we had observed between patients might be the result of differing degrees of infection in the cervical mucosa in individual patients.

The aim of this study was to see if certain clinical factors might be caused by, or be the cause of, high-grade rather than low-grade infection. In particular it seemed essential to consider (a) the physical state of the cervix—that is, whether or not mucopus or ectopy were present; (b) hormonal effects on cervical epithelium—that is, whether or not the patient was using oral contraceptives; (c) if chlamydial infection might be enhanced opportunistically by concurrent infections, especially gonorrhoea; and (d) the age of the patient.

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Accordingly, clinical and laboratory data were compared in the total of 580 women from whom *C trachomatis* had been isolated in this laboratory between January 1975 and December 1978. This series excludes only women with postpartum infection and those who had recently received antibiotic treatment, but it was not restricted to women with a definite history of sexual contact with men with NGU and did not exclude women with concurrent gonorrhoea.

### Patients and methods

#### STUDY POPULATION

The 580 women consecutively found to have chlamydial infection on laboratory examination were all patients attending the sexually transmitted disease (STD) clinics at the Liverpool Royal Infirmary or the Royal Liverpool Hospital or who had been referred to the Liverpool Women's Hospital from various sources because of suspected genital tract infections. Only women whose infections were first diagnosed in the puerperium, or who had been given chemotherapeutic agents within the previous four weeks, were excluded.

Clinically, the group consisted of 70 women from a consecutive series of sexual contacts of men with NGU, whose clinical findings have been compared with those of contacts without chlamydial infection,<sup>3</sup> together with 49 infected women examined in a consecutive unselected series of patients attending a single STD clinic of the Royal Infirmary on specified days (Davies *et al*, unpublished data).

The remaining 341 women were not seen consecutively in the clinics; in fact, they had often been deliberately selected for laboratory investigation because they did show certain clinical signs of genital infection, or because they were contacts of men with NGU or with gonorrhoea, or for various other reasons which made it important to confirm or exclude chlamydial infection in their differential diagnoses and to assess its severity if present. This element of clinical selection does not affect the proposed correlation of laboratory findings with the clinical features presented by the patients, since the investigation is based on the laboratory's total number of chlamydia-positive specimens, unselected and completed in advance of the clinical data being known. Obviously, the proportion of women with such features as cervical mucopus, cervical ectopy, or concurrent gonorrhoea cannot be taken as comparable with that in clinically unselected patients seen consecutively in studies designed for clinico-epidemiological purposes.<sup>1,3</sup>

#### CLINICAL EXAMINATION

Examination of the patients' clinical signs was made

and recorded in the standardised pattern described earlier.<sup>3</sup>

#### COLLECTION OF SPECIMENS

Swabs for chlamydial isolation were taken only by medical staff of the clinic experienced in the standardised technique previously described,<sup>5</sup> which ensures that both the contents of the endocervical canal and the whole of any area of ectopic columnar epithelium present are sampled.

Swabs for *Neisseria gonorrhoeae* isolation and investigations for syphilis, trichomoniasis, or yeast infections were performed on all patients as previously described.<sup>3</sup>

#### LABORATORY INVESTIGATIONS

The procedures for the isolation of *C trachomatis* and subsequent counts of the total number of chlamydial inclusions recovered have been previously described in detail.<sup>2,4-6</sup> Briefly, particular attention was paid to the quantitative reproducibility of the isolation procedure—for example, immediate immersion of the swab into fluid transport medium, rapid transport to the laboratory and inoculation usually 2-4 hours after the swab was taken but occasionally after overnight storage at 4°C, followed by inoculation of a standardised amount of homogenised swab material with centrifuge-assistance on to McCoy cell coverslip monolayers of constant age and cell density. For the first 260 swabs, untreated McCoy cells were used, but cells treated with cycloheximide were used for the final 200 specimens, and both methods were used simultaneously for the remaining 120 specimens. Cycloheximide treatment has been adopted because chlamydial inclusions can be identified more rapidly and easily than in fresh cells; the total number of inclusions recovered was consistently higher in treated cells, but rarely by more than twofold, so that the log frequency distribution of counts did not vary significantly between the two methods.

In all cases, the inclusion counts recorded represent the primary inoculation of the first cervical swab to be taken from each patient during her attendance in the clinic. All swabs were allocated serial laboratory numbers, and isolation and inclusion counting procedures were completed before the patient's identity and clinical features were reviewed in the laboratory.

#### STATISTICAL METHOD

The  $\chi^2$  test was used for statistical comparison.

### Results

#### INCLUSION COUNTS AND CLINICAL FEATURES

The wide range of inclusion counts found on culture of the primary cervical swabs for the whole group of

TABLE I *Distribution of chlamydial inclusion counts and clinical factors in 580 women with C trachomatis infection of the cervix*

iwc	No of women	% of women* with positive clinical factor				
		Mucopus	Ectopy	OC	Gonorrhoea	Aged <20 years
<10	84	36	58	49	38	23
10+	112	36	63	50	35	24
100+	178	44	67	54	44	26
1000+	133	69	79	62	44	35
10 000+	73	66	75	66	34	40
Total No of women	580	290	402	306	232	168

iwc = No of inclusions per whole coverslip McCoy culture inoculated with cervical swab material

OC = Oral contraceptives

\*In each inclusion-count group

580 women is shown in table I. Although the counts ranged from only one inclusion per whole coverslip (iwc) to >75 000 they showed a substantially normal distribution with a median count of 450 inclusions; 476 (85.5%) of the swabs yielded >10 iwc and 207 (36%) gave  $\geq 1000$  iwc.

The distribution of the main clinical factors is also outlined in table I. The overall incidence of mucopus, ectopy, and gonorrhoea was high, as was to be expected by the clinical selection of these women. Nevertheless, certain clinical factors were not uniformly distributed among the whole group.

The greater the inclusion count, the greater was the proportion of patients who were under 20 years of age, showed cervical ectopy or mucopus, or were taking oral contraceptives. There did not however seem to be any significant correlation between the height of the inclusion count and concurrent gonorrhoea.

These observations suggest that the degree or severity of infection in the cervical mucosa varied according to the presence or absence of certain clinical factors affecting the cervix. To evaluate their

interactions more precisely the clinical features of women at the opposite ends of this frequency distribution of inclusion counts—that is, those with low counts of <100 iwc and those with high counts of  $\geq 1000$ —were compared (table II).

#### *Cervical mucopus/concurrent gonorrhoea*

The amount of chlamydial growth in the cervical mucosa was significantly greater in women with mucopus than in those without (table II;  $\chi^2_1 = 41.1$ ,  $P < 0.001$ ). A heavy chlamydial infection could be the actual cause of the mucopus but alternatively some other clinical factor could be the cause of both the mucopus and the high chlamydial count. For the latter reason, the fact that 143 (35%) of the 403 women had gonorrhoea concurrently with their chlamydial infection had to be taken into account. Gonorrhoea itself is obviously a common cause of cervical mucopus and chlamydial infection might possibly be enhanced opportunistically in tissues already being damaged by *N gonorrhoeae*.

However, high chlamydial counts were not significantly more frequent in women with associated gonorrhoea than in women with chlamydial infection alone ( $\chi^2_1 = 1.33$ ,  $P > 0.1$ ) (table II). Furthermore, the

TABLE II *Distribution of clinical factors in relation to low or high inclusion counts*

Clinical factor	No of women with inclusion counts		Total
	Low (<100 iwc)	High (>1000 iwc)	
Mucopus +	70	140	210
Mucopus -	126	67	193
Gonorrhoea +	64	79	143
Gonorrhoea -	132	128	260
Ectopy +	120	160	280
Ectopy -	76	47	123
Oral contraception +	94	130	224
Oral contraception -	102	77	179
Age (years) <20	38	76	114
Age (years) >20	158	131	289
Total (%)	196 (34)	207 (36)	403

+ Present - absent

iwc = inclusions per whole coverslip (10 mm diam)

TABLE III *Prevalence of cervical mucopus in relation to inclusion counts in women with or without concurrent gonorrhoea*

Clinical factor	No of women with inclusion counts		Total
	Low (<100 iwc)	High (>1000 iwc)	
Gonorrhoea -			
Mucopus +	45	82	127
Mucopus -	87	46	133
Gonorrhoea +			
Mucopus +	25	58	83
Mucopus -	39	21	60
Total	196	207	403

+ Present - absent

iwc = inclusions per whole coverslip (10 mm diam)

association between mucopus and high chlamydial counts was not dependent on a concomitant infection with *N gonorrhoeae*, as was shown by evaluating separately the data in the groups of women with and without gonorrhoea (table III).

Mucopus was present in a high proportion of all the women studied (210/403, 52%); this could be partly due to the clinical selection of the cases before referral for laboratory diagnosis. However, the proportion of cases with mucopus was similar in women with chlamydial infection alone (127/250, 65%) and in chlamydia-positive women with concurrent gonorrhoea (83/143, 70%) ( $\chi^2 = 3.13$ ,  $P > 0.05$ ). Moreover, there was a significant association between the presence of mucopus and high inclusion counts whether or not the woman had a chlamydial infection alone ( $\chi^2 = 23.36$ ,  $P < 0.001$ ) or concomitant gonorrhoea ( $\chi^2 = 17.14$ ,  $P < 0.001$ ).

#### Cervical ectopy/oral contraceptives

When these clinical factors were considered separately (table II) each showed a significant association with the height of the inclusion count. There was little difference between simple ectopy or hypertrophic ectopy in relation to inclusion counts and both have been combined for statistical analysis. Because of the clinical selection cervical ectopy was present in over two-thirds of the 403 women studied (table II). However, 57% of these women had high inclusion counts compared with 38% of women without ectopy ( $\chi^2 = 12.3$ ,  $P < 0.001$ ). Oral contraceptives were being used by 56% of the 403 women and 58% of these patients had high inclusion counts compared with 43% of women not taking oral contraceptives ( $\chi^2 = 8.98$ ,  $P < 0.01$ ).

However, cervical ectopy and oral contraceptives were obviously not independent variables, since only 106 (38%) of the 280 women with ectopy were not taking oral contraceptives and only 50 (22%) women using oral contraceptives did not have cervical ectopy. In fact, the association between the use of oral contraceptives and the presence of ectopy was highly significant ( $\chi^2 = 47.08$ ,  $P < 0.001$ ); thus these two clinical factors probably interact in their effect

on the height of the inclusion count, as they have previously been shown to affect the incidence of chlamydial infection.<sup>3</sup> To assess this interaction, the four possible combinations of the two factors were further analysed (table IV):

1. In patients without cervical ectopy, high counts were found in 32% of women not taking, and in 48% of women taking, oral contraceptives. The effect of oral contraceptives alone was not significant ( $\chi^2 = 3.42$ ,  $P > 0.05$ ).
2. In patients with cervical ectopy, high counts were found in 51% of women not taking, and in 61% of women taking, oral contraceptives. The effect of oral contraceptives alone was not significant ( $\chi^2 = 2.67$ ,  $P > 0.1$ ).
3. In patients with oral contraceptives, high counts were found in 48% of women without ectopy and in 61% of women with ectopy. The effect of ectopy alone in these women was not significant ( $\chi^2 = 2.66$ ,  $P > 0.1$ ).
4. In patients without oral contraceptives, high counts were found in 32% of those without ectopy and in 51% of those with ectopy. This effect of ectopy alone was significant ( $\chi^2 = 6.67$ ,  $P = 0.01$ ).

When the patients with neither ectopy nor oral contraceptives were compared with those with both ectopy and oral contraceptives, the difference in the proportion of cases with high inclusion counts, 32% and 61% respectively, was significant ( $\chi^2 = 17.8$ ,  $P < 0.001$ ).

These results suggest that ectopy and oral contraceptives acting jointly are likely to be associated with high chlamydial counts and that ectopy alone can result in high counts even if the ectopy has not been produced by, or the cervical mucosa has not been acted upon by, oral contraceptives. The effect of oral contraceptives on chlamydial counts appears to be indirect by way of its capacity to produce ectopy. When these possible effects are discounted, by dividing the women into groups (table IV), oral contraceptives appear to have no significant direct effect on the degree of chlamydial infection.

TABLE IV Interaction of cervical ectopy, oral contraceptives, and inclusion counts

Clinical factors		No of women with inclusion count			% with high count
Ectopy	OC	Low (<100 iwc)	High (>1000 iwc)	Total	
-	-	50	23	73	32
-	+	26	24	50	48
+	-	52	54	106	51
+	+	68	106	174	61
Total		196	207	403	

+ Present - absent

iwc = inclusions per whole coverslip (10 mm diam)

OC = Oral contraceptives

### Age factor

Of the total of 403 women with chlamydial infections, 114 (28%) were under 20 years of age (table II); of these, 76 (67%) had high inclusion counts compared with 131 (45%) of the 289 women over 20 years of age ( $\chi^2 = 14.9$ ,  $P < 0.001$ ).

Despite this significant difference in the degree of infection between the under-20 age group and older women, no significant differences emerged in any of the other clinical features (table V).

### Other clinical features

There were 119 women with one or more previous children and 22 pregnant women in the group examined; 36 women had associated trichomonal infections, 89 were infected with yeasts, and 31 had signs or symptoms suggesting salpingitis. There was no evidence that the frequency of high chlamydial counts was greater in these women than in women without these clinical features. However, the small numbers limit conclusive statistical analysis.

No significant difference was found in the proportion of women with high inclusion counts in successive weeks of the menstrual cycle. In the group of 403 women studied, 22 were pregnant, 32 had irregular periods or amenorrhoea, and in 20 the date of the last period was not recorded. In the 329 (82%) women with a regular cycle of 26-30 days, the month was divided into four weeks starting from the first day of menstruation, and the cycle-week in which the primary cervical swab for *C trachomatis* isolation was taken was recorded against the inclusion count obtained on culture (table VI).

### Discussion

Current tissue culture methods for isolating *C trachomatis* from cervical swabs of infected women provide a quantitative means of monitoring the reproducibility and sensitivity of the procedures themselves in continued diagnostic use<sup>2,4</sup> and of detecting differences in the degree of infection between individual patients, since the number of inclusions which develop in McCoy cell culture is directly proportional to the number of viable chlamydial elementary bodies inoculated into the culture.<sup>7</sup>

TABLE V Distribution of clinical features in relation to age

Clinical feature	% of women	
	<20 years	≥20 years
Mucopus	51	53
Gonorrhoea	40	39
Ectopy	75	68
Oral contraceptives	52	57

In the present series of 580 women with chlamydial genital infections from whom a primary cervical swab was taken for laboratory culture before they had been given chemotherapeutic agents, the inclusion counts were normally distributed with a median value of 450 iwc, but the range of counts obtained was wide, with 73 (13%) of the group having counts of  $\geq 10\,000$  iwc whereas 84 (14%) had counts of  $< 10$  iwc. When the inclusion counts in the individual patients were related to the clinical features found at the time the swab was taken, the degree of infection (indicated by the inclusion count) apparently varied according to the presence or absence of cervical mucopus or ectopy, the use of oral contraceptives, and the age of the patient.

The increase in inclusion counts in women with cervical mucopus may be due to the following: (a) the greater the amount of secretion in the cervical canal the greater is the amount that might be collected on the swab, and hence the greater the number of elementary bodies available to infect the McCoy coverslip culture; (b) the presence of mucopus might non-specifically enhance the sensitivity of McCoy cells to chlamydial infection, resulting in a higher proportion of the potentially infective particles inoculated successfully forming inclusions in McCoy cells; (c) mucopus may be merely an indicator of another concurrent infection, especially gonorrhoea, which might be capable of enhancing the chlamydial infection—that is, *C trachomatis* might thus show the characteristics of a secondary opportunistic invader; and (d) mucopus might in fact be the direct result of chlamydial infection and thus occur most frequently in those with the greater degree of infection, as indicated by the inclusion count.

In our experience the thick tenacious nature of the mucopus often results in rather less than more cervical secretion being obtained per swab and limits the amount of homogenisation of material from the swab before tissue culture inoculation. Suspensions of cervical mucopus from patients with STDs but without current chlamydial infection are slightly but consistently inhibitory to the growth of laboratory strains of *C trachomatis* in McCoy cultures (Hobson and Lee, unpublished data).

TABLE VI Inclusion counts in relation to menstrual cycle

	Week of cycle*				Total
	1	2	3	4	
No of patients	60	112	79	78	329
No with high count ( $> 1000$ iwc)†	31	60	40	42	173
% with high count	52	54	51	54	53

\*At the time of primary cervical swab

†Inclusion count in primary cervical swab

Concurrent infection with trichomonads, yeasts, or *N gonorrhoeae* appeared to have no effect on the degree of chlamydial infection. It is interesting however that women with gonorrhoea have previously been found<sup>8,9</sup> to have a higher rate of chlamydial cervical infection than have contacts of men with NGU or attenders of STD clinics without gonorrhoea. This incidence may be increased because coincidental infections in the more promiscuous STD clinic patients, who must frequently be exposed to both types of infective agent, are highly likely rather than because gonorrhoea predisposes to or stimulates a previously latent or low-grade chlamydial infection. Furthermore, women who had concomitant infections did not develop mucopus more frequently than did women with chlamydial infections alone; in both groups the presence of mucopus was associated with high chlamydial inclusion counts. These results thus suggest that the presence of mucopus in a woman with gonorrhoea may, in certain cases, be the result of an associated chlamydial infection rather than due to the gonococcal infection alone.

There is a highly significant association between the chlamydial isolation rate and the incidence of cervicitis in contacts of men with NGU,<sup>3,10</sup> and treatment of the chlamydial infection is quickly followed by disappearance of mucopus.<sup>3</sup> The present results support and extend these findings—that is, *C trachomatis* can cause endocervicitis and the greater the degree of infection the more likely are the signs of endocervicitis to be present.

In contacts of men with NGU seen in Liverpool<sup>3</sup> a higher rate of chlamydial infection was found when both cervical ectopy and contraceptive steroids were present than when either occurred alone or when neither was present. All the infected women from this smaller study group<sup>3</sup> are included in the present larger and more varied series, which shows that not only the incidence but also the degree of chlamydial infection was greater in women with ectopy and contraceptive steroids than in women with neither. Oral contraceptives alone did not appear to have any influence on the degree of infection but, in contrast, the inclusion counts were significantly higher in those with ectopy alone—even in the absence of oral contraceptives—than in women without ectopy. Since the site of the squamo-epithelial junction is determined during fetal life,<sup>11</sup> infection with *C trachomatis* cannot be a cause of cervical ectopy, although infection may convert a small area of simple ectopy to a larger area of hypertrophic ectopy as a result of oedema and congestion.<sup>3</sup>

The most likely explanation of the significant association between the presence of ectopy and both an increased rate of infection<sup>3</sup> and an increase in the

degree of that infection demonstrable in tissue culture is simply that the greater the area of columnar epithelium exposed on the vaginal aspect of the cervix the greater is the chance that infection will be acquired after coitus with an infected man, the greater is the number of susceptible columnar cells available for progressive growth cycles of the infective agent, and the greater is the area that can be sampled by taking swabs.

The role of oral contraceptives is more difficult to determine and is almost certainly multifactorial. The frequency and size of cervical ectopy is itself increased by the use of contraceptive steroids,<sup>12,13</sup> and thus the chlamydial inclusion count is likely to be significantly increased if oral contraceptives are considered overall, but much of this increase may be the result of the ectopy associated with the use of oral contraceptives and not uniquely due to the drugs. However, oral contraceptives may possibly also have direct hormonal effects on columnar epithelium in vivo which increases the chance that a highly productive infection with *C trachomatis* will develop. Contraceptive steroids have a progestational effect, producing a large outflow of alkaline mucus from the endocervical glands.<sup>12</sup> The addition of steroids to McCoy cell cultures increases their susceptibility to infection with laboratory strains of *C trachomatis*, resulting in an increase in the number of infective progeny produced by the inoculation of a given number of elementary bodies.<sup>14</sup> Thus, as suggested by the present findings, the hormonal effects of oral contraceptives would be seen most clearly when acting in conjunction with cervical ectopy, especially since the action of these steroids increases the bulk of the cervix and produces eversion.<sup>12</sup> However, in the present series of women there was no significant association between the height of the inclusion count and other hormonal influences, either in the normal menstrual cycle or in pregnancy, although a much larger series of women would need to be examined to confirm the latter.

A further feature needing more extensive investigation is the highly significant association between the age of the patient and the degree of her infection in the present series of women. This association might possibly reflect anatomical and physiological, including hormonal, differences between women under 20 years of age and older women, but the present findings do not suggest that the increased inclusion counts are the results of a greater use of oral contraceptives or of a greater frequency of cervical ectopy than in women between 21–45 years of age. However, the exact area of ectopy was not measured in these women. The size of cervical ectopy diminishes with age owing to metaplasia, whereby columnar epithelium is gradually replaced by

squamous epithelium, and this process is often initiated at puberty or in the first pregnancy.<sup>11</sup> Thus, possibly the area of ectopic columnar cells, rather than the frequency of ectopy, may vary between young and older women to influence their differing degrees of chlamydial infection.

It is also possible that this age difference may have an immunological basis. In girls aged between 14-20 years a greater proportion of the infections are probably the first chlamydial infections to which they have been exposed than is the case in older women with a longer period of sexual activity. Thus, the replication of *C trachomatis* may be less likely to be brought quickly under control by humoral or cervical antibody or by cell-mediated immune responses in young women than in older women with a greater chance of previous immunological experience. Humoral antibody against *C trachomatis* is frequent and persistent in women attending STD clinics and its frequency increases with age.<sup>15-17</sup> Although it is not yet clear that antibody acquired by previous experience will prevent infection, the possibility that it might modify the degree of infection and hence the clinical signs associated with high-inclusion count infection merits further study.

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